A POLAROGRAPHIC MICRO-METHOD FOR THE DETERMINATION OF BLOOD CHLORIDE*

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ABSTRACT

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OBJECT

To devise a simple, rapid and accurate technique for the determination of chloride concentration in small volumes of blood to facilitate investigations of electrolyte changes in human subjects and laboratory animals.

RESULTS AND CONCLUSIONS

A polarographic micro-method for the determination of chloride concentration in 0.05 ml. of whole blood, plasma, and serum has been developed. Its accuracy as measured by reproducibility, addition studies, and comparison with a standard macro-method is within 1 per cent. The equipment used for the determination is not complicated and only a short time is required for analysis.

The method has been found to be especially suited for frequent serial chloride determinations in small laboratory animals, because the small volume of blood necessary for the test does not produce electrolyte changes attending excessive blood loss.

RECOMMENDATIONS

The method should be of value in the determination of blood chloride concentration in human subjects undergoing physiological tests because it obviates the necessity for frequent venipunctures, as sufficient blood for analysis can be drawn from a fingertip.

The apparatus used could be modified so as to be compact and sturdy, suiting it for use in investigations in the field.

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I. INTRODUCTION

Because of the inherent sensitivity, rapidity and simplicity of the polarographic method for the quantitative analysis of inorganic ions, the present study was undertaken to determine the feasibility of the method for the accurate determination of chloride concentration in small volumes of whole blood, serum and plasma.

Kolthoff and Miller (1) have shown that the concentration of the chloride ion in a solution can be determined by measurement of the diffusion current of the anodic depolarization wave produced at the dropping mercury electrode. Using 0.1 N. potassium nitrate as a supporting electrolyte, they found that the diffusion current was directly proportional to chloride concentrations between 10^{-4} and 2×10^{-3} equivalents per liter.

The electrode reaction is:

$$2\ Hg + 2\ Cl^- \longrightarrow Hg_2Cl_2 + 2\ e$$

II. EXPERIMENTAL

A. Apparatus and Methods

A manual apparatus (Figure 1) similar to that described by Kolthoff and Lingane (2) was used in the determinations described in this report. A potentiometer with a range of 1.5 volts was used as a source of E.M.F. The diffusion current was measured with a reflecting galvanometer and scale. The sensitivity of the galvanometer was adjusted by means of a shunt to approximately 0.013 μa/mm. Several capillaries were used, with drop times of between 2 and 4 seconds.

A compact electrolysis cell-reference electrode assembly was developed which greatly facilitated the operation of rinsing the cell and removal of solution following analysis (see Figure 1). The inclined surface of the agar bridge (0.1 M.KNO_3 in 3% agar) allows complete drainage of cell contents through the side arm, and the cell may be filled without removing it from the electrode assembly. Thus, the capillary, cell and saturated mercurous sulfate reference electrode assembly constitute one unit which does not need to be disassembled for filling or cleaning, once it has been set up. A water jacket, holding about 100 ml. is placed around the analysis cell to reduce the rate of temperature change that can occur during a series of analyses. The exact temperature of the cell is unimportant, since the unknown solutions are compared with standards. As long as the temperature change during the course of an analysis does not exceed 0.5°C., the error due to this change will be less than 1 per cent (2).

It is not necessary to remove dissolved oxygen from solutions to be analysed since oxygen is reduced at the dropping mercury electrode at potentials negative to the chloride wave. The galvanometer used to determine

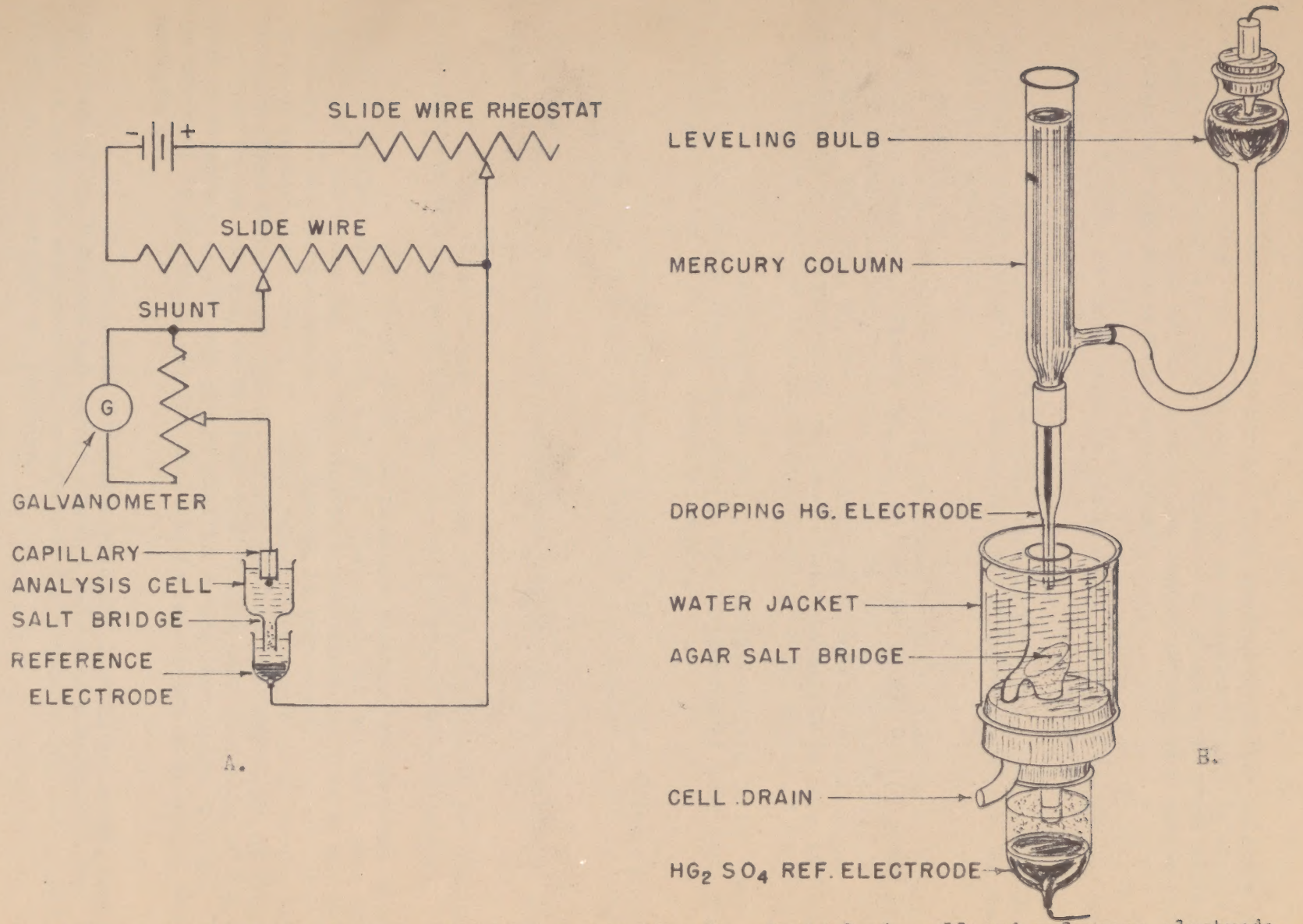

FIG. 1. A. Schematic diagram of polarographic circuit, electrolysis cell and reference electrode.

B. Detailed diagram of dropping mercury electrode-electrolysis cell--reference electrode assembly.

diffusion current need not be calibrated since chloride solutions of known concentrations are used as standards.

Since the diffusion current is proportional to the chloride ion concentration only over a limited range, it is necessary to determine the diffusion current from chloride in blood at a dilution of about 1:100. Phosphotungstic acid serves the dual purpose of acting as a supporting electrolyte and as a protein precipitant.

1. The reagents used in the preparation of the sample are as follows:

a. Stock standard potassium chloride solution. 0.2 M. Dried, analytical reagent grade, potassium chloride, 14.92 gm. diluted to one liter with distilled water.

b. Working standard potassium chloride solutions. Transfer to 100 ml. volumetric flasks 55, 50, 45, 40 and 35 ml. of the stock standard potassium chloride solution. Dilute to 100 ml. to prepare standards containing 110, 100, 90, 80 and 70 milliequivalents of chloride per liter respectively. At least three of these standards should be used to include the expected chloride concentration in the series of bloods being analysed.

c. Phosphoric acid solution, approximately 0.15 M. Into a one liter volumetric flask, transfer 10 ml. of phosphoric acid (sp. gr. 1.7) and dilute to the mark with distilled water.

d. Sodium tungstate solution, 3.0 per cent. Three gm. of reagent grade sodium tungstate are dissolved and diluted to 100 ml. with distilled water.

2. The following technique was used in the preparation of the sample:

a. Into as many test tubes as will be required for the unknown and standard solutions, deliver 4.0 ml. of phosphoric acid solution.

b. The blood sample or standard solution is measured in a pipette calibrated to contain 0.05 ml. The sample is delivered into the phosphoric acid solution and the pipette washed clean by drawing up the diluting fluid several times. The pipette is then blown out and carefully drained against the side of the tube. Care is required in measuring these small samples to see that the tip of the pipette is wiped clean of fluid adhering to the outside, at the same time taking care that none of the sample is drawn out of the end of the pipette during the wiping process. The measurement of the sample is the limiting factor in the accuracy of the procedure.

c. Of the sodium tungstate solution 1.0 ml. is added, and the contents of the tube are thoroughly mixed. The tubes containing blood samples are then stoppered and centrifuged for approximately one minute at 2000 r.p.m. to precipitate proteins.

d. The supernatant solutions are introduced into the analysis cell. When 0.02 ml. samples are used, the procedure is exactly as above, except 1.6 ml. of phosphoric acid solution and 0.4 ml. of the sodium tungstate solution are used.

The cell is rinsed once with the solution to be analysed, refilled and the minimum and maximum galvanometer deflections are recorded. The mean deflection for each of the standard solutions is plotted on coordinate paper against chloride ion concentration and this plot is used as a calibration curve (see Figure 2).

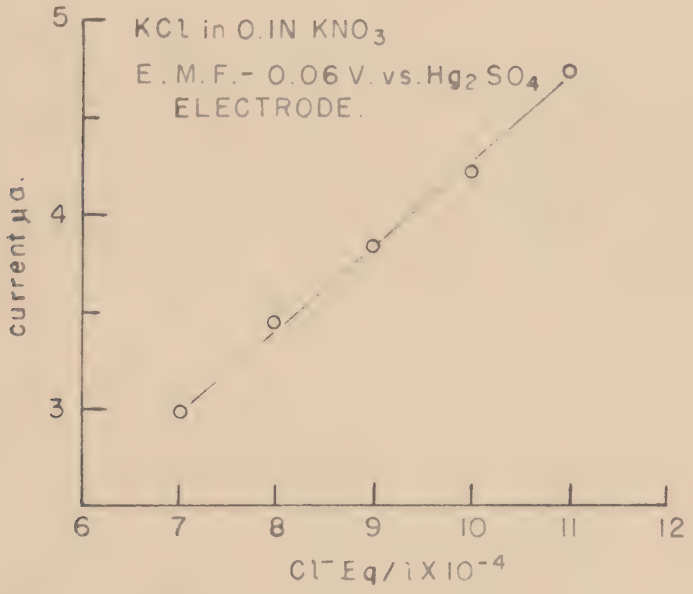

FIG. 2 - DIFFUSION CURRENT PLOTTED AGAINST Cl^- ION CONCENTRATION.

It is not necessary to plot the entire current-voltage curve for each test solution. All of the determinations are made at a single, constant voltage setting. This voltage is selected by plotting current voltage curves for a series of chloride solutions of known concentration of a sufficient range to include chloride values to be encountered. A voltage setting is selected at which the increment of diffusion current is directly proportional to the increment of chloride concentration. This voltage will vary with the kind of supporting electrolyte used. In the investigations described in this report, the applied voltage was found to be from 0 to -0.06 volts against the saturated mercurous sulfate electrode, depending on the supporting electrolyte used. As long as the diffusion current remains a linear function of chloride ion concentration, additional current voltage curves need not be determined. In the current-voltage curve shown in Figure 3, the voltages between the lines labeled V min. and V max. are suitable for measurement of diffusion currents.

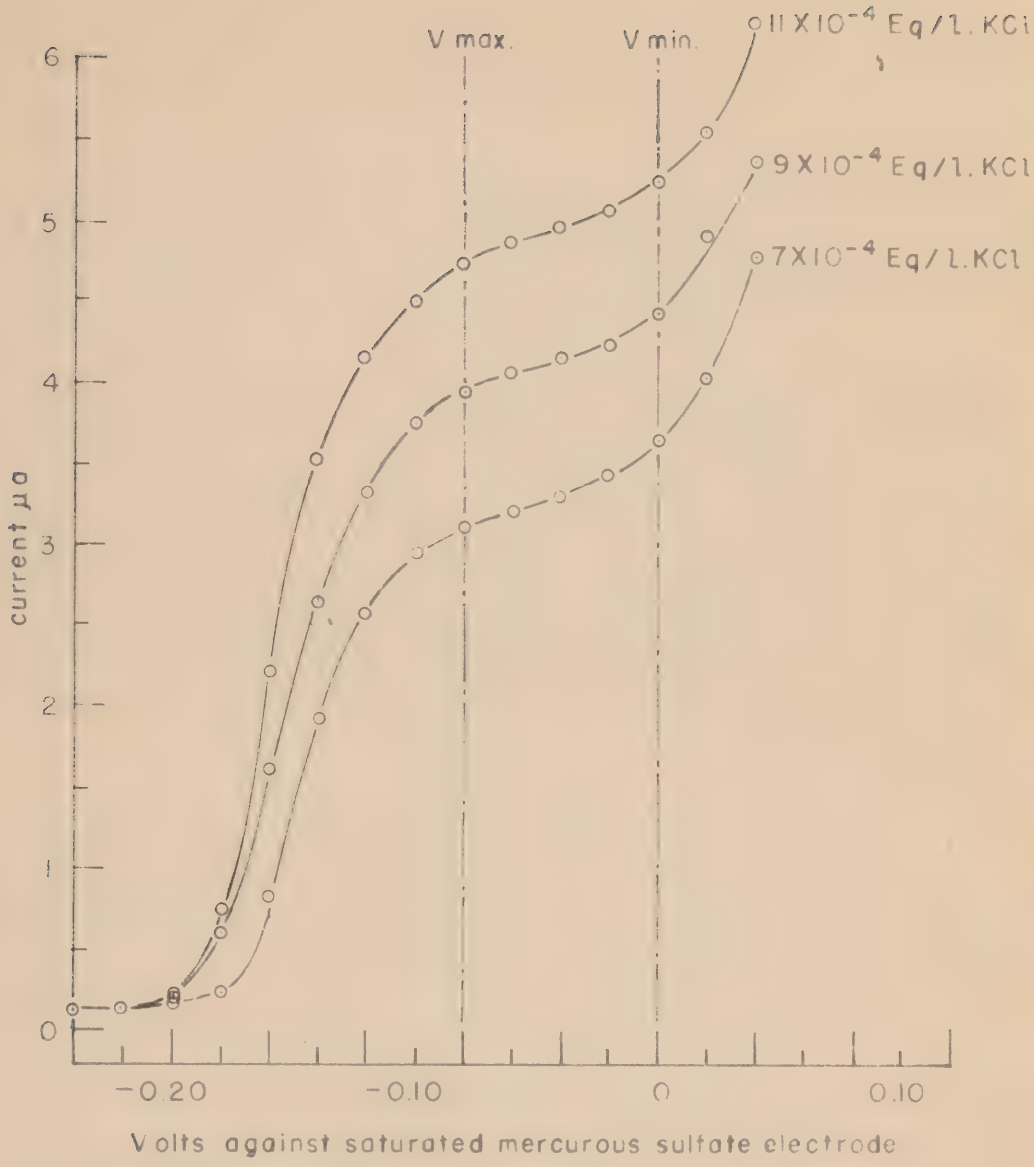

FIG. 3 CURRENT-VOLTAGE CURVES OF KCl - 7, 9 & 11 X 10^{-4} Eq/l. IN 0.1 N KNO_3.

B. Results

The accuracy of the method has been evaluated by the following studies:

1. The proportionality of the diffusion current to chloride ion concentration. It can be seen from Figure 2 that there is a precise and direct proportionality of diffusion current to chloride ion concentration.

2. The agreement of duplicate determinations. The agreement of duplicates was essentially the same whether the determination were made on whole blood, plasma or serum. In a series of 2[illegible] determinations in duplicate on whole blood, plasma and serum, the average difference of duplicates using the phosphotungstic acid procedure was 0.57 per cent.

3. Recovery of a known quantity of chloride added to plasma. Rat blood was used with a solution of ammonium and potassium oxalates as an anticoagulant. The plasma was separated and the chloride was determined in duplicate using 0.05 ml. samples prepared by the phosphotungstic procedure. Another set of determinations was carried out on these samples using a phosphoric acid solution containing 2.0 ml. of 0.1 N. HCl per liter, in place of the usual phosphoric acid diluting solution. This is equivalent to the addition of 16.0 mEq/L of chloride to the original plasma. The quantity of added chloride was 2[illegible].4 micrograms in 5.05 ml. The results of this experiment are shown in Table 1.

TABLE 1

RECOVERY OF A KNOWN QUANTITY OF CHLORIDE ADDED TO PLASMA

Plasma Chlorides (average of duplicates) mEq/L	Chloride Added mEq/L	Total Chloride Found mEq/L	Chloride Recovered mEq/L
94.8	16.0	110.6	15.8
95.5	16.0	110.0	14.5
94.8	16.0	110.4	15.6
101.5	16.0	118.9	17.4
106.3	16.0	123.3	17.0
101.2	16.0	11[illegible].0	16.8

4. Comparison of chloride values obtained by the polarographic micro-method with values determined by a standard volumetric macro-method. A series of 2[illegible] determinations was carried out in duplicate using the phosphotungstic acid technique and compared with the iodate titration method of Sendroy as modified by Van Slyke and Hiller (3) using 1 ml. samples. The blood was obtained from white rats and prepared as before. Since the agreement with the iodate titration method was essentially the same for plasma, serum and whole blood, the results for the entire series are summarized in Table 2.

TABLE 2

COMPARISON OF CHLORIDE VALUES OBTAINED BY THE POLAROGRAPHIC MICRO-METHOD WITH VALUES DETERMINED BY THE SENDROY (MACRO) METHOD

	Phosphotungstic Acid Procedure (28 Determinations)
Mean difference from Sendroy Value	0.99 mEq
Difference, 1 mEq or less	64%
Difference, 1.5 mEq or less	75%
Difference, 2.0 mEq or less	86%

III. DISCUSSION

The value of a biochemical analytical technique depends on its accuracy and simplicity. The accuracy of the polarographic micro-method has been evaluated in the preceding section. Since current voltage curves are not plotted in this procedure, simple apparatus may be used and the diffusion current may be determined immediately after introduction of the sample into the analysis cell. It has been found that this method is considerably faster than other macro and micro procedures for the determination of chloride.

As this method was originally developed, a modification of the Somogyi zinc sulfate-barium hydroxide method (4) was used for the precipitation of proteins. The accuracy of this method was found to compare favorably with the phosphotungstic acid procedure, but the necessity of exactly matching the strengths of the two reagents made this procedure technically more difficult.

While the above investigations were in progress, the work of Schönholzer (5) on the polarographic determination of chlorides in biological materials came to the attention of the authors. In this procedure, proteins are not removed from the solution to be analysed, but the sample is diluted in 0.1 N. sulfuric acid which acts as the supporting electrolyte. Because of the simplicity of the method, its accuracy was determined carefully. A series of determinations in duplicate was carried out on 9 serums and 9 whole bloods together with parallel determinations on the same samples by the Sendroy macro-method and the polarographic micro-method using the phosphotungstic acid procedure. Since the agreement of results with serum and whole blood were essentially the same, the results from the entire series are summarized together. The average difference between duplicates was 1.14 per cent. The comparison with the parallel series of phosphotungstic acid procedure can be seen in Table 3.

TABLE 3

COMPARISON OF CHLORIDE VALUES OBTAINED BY THE PHOSPHOTUNGSTIC ACID AND SULFURIC ACID POLAROGRAPHIC METHODS WITH VALUES DETERMINED BY THE SENDROY METHOD, 18 PARALLEL DETERMINATIONS

	Phosphotungstic Acid Procedure	Sulfuric Acid Procedure
Mean difference from Sendroy Value	0.93 mEq	1.48 mEq
Difference, 1 mEq or less	67%	28%
Difference, 1.5 mEq or less	78%	50%
Difference, 2.0 mEq or less	83%	78%

The dilution of the sample with 0.1 N. sulfuric acid as described by Schönholzer is the simplest of the procedures and because of this, is the one to be recommended for material which contains little or no protein. However, the increased accuracy attained by the removal of protein seems sufficient to warrant the additional step used in the phosphotungstic acid procedure when determinations are done on whole blood, serum or plasma.

The reason for the decreased accuracy of the sulfuric acid method has not been determined. It appears that the presence of protein is undesirable in a solution to be used for polarographic analysis of chloride, since the unknown solutions differ to this extent from the standard chloride solutions used for calibration, and since more reproducible results are obtained when proteins are removed.

There are a number of substances which, if present in an unknown solution, will add to the diffusion current due to chloride (Kolthoff and Miller (1)). These substances do not naturally occur in blood but may be present as a result of their administration. The only such substances likely to be found are thiosulfate, thiocyanate, and bromide. When these are known to be present, the polarographic method as described here cannot be used.

IV. CONCLUSIONS

The concentration of chloride ion in 0.05 ml. of whole blood, serum or plasma can be measured with an accuracy of approximately 1 per cent by means of the polarograph. The determination is more accurate following the removal of proteins. The method is rapid and simple.

V. RECOMMENDATIONS

The method should be of value in the determination of blood chloride concentration in human subjects undergoing physiological tests because it obviates the necessity for frequent venipunctures, as sufficient blood for analysis can be drawn from a fingertip.

The apparatus used could be modified so as to be compact and sturdy, suiting it for use in investigations in the field.

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